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Microfluidic device for refractive index measurement of fluid sample

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Abstract

This paper represents a novel microfluidic device for simple refractive index (RI) measurement of fluid sample with sub microliter volume. The defocusing imaging technique using a three pinhole aperture plate is implemented for the refractive index measurement. For the self-calibration, the microfluidic device has a measurement region and a self-calibration region, and both regions are designed to be captured on single image frame. Thus, the refractive index of a sample fluid is automatically calculated with the self-calibration. For the demonstration of the proposed device, standard refractive index liquids with the refractive indices of 1.300, 1.400, 1.500, 1.600 and 1.700 are used. The measured refractive index has the maximum deviation of 0.0036 (0.24%) from the standard refractive index values.

Keywords: Refractometer; Microfluidic device; Defocusing imaging; three pinhole; self-calibration; Refractive index measurement;

1. Introduction

The refractive index of the materials is an essential property for all optical applications including composition

Nomenclature

d	diameter of a circle circumscribed around three pinholes
n	refractive index
D	diameter of a circle circumscribed around three separated spot images

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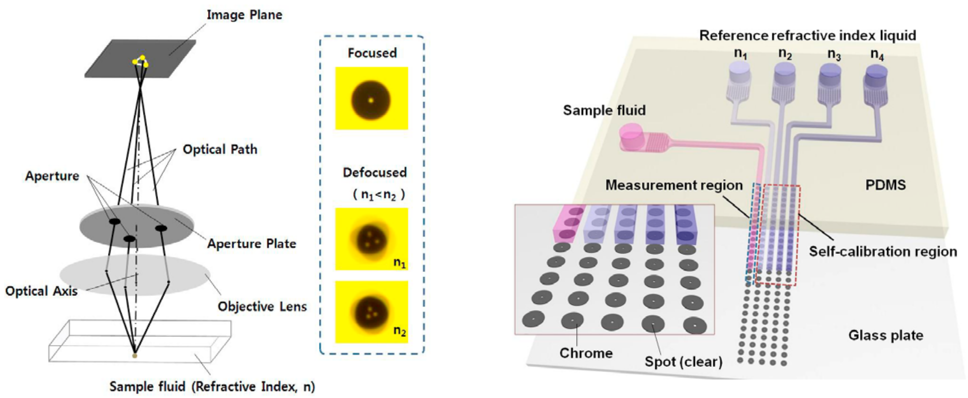
analysis and quality monitoring in pharmaceutical and food production [1-2]. Particularly, the refractive index measurement of biofluid samples in many cases is challenging due to small sample volume and thus, a device, which is able to provide accurate refractive index value even for small sample volume, is necessary. Furthermore, most developed refractometry has complicated system configuration so that it is beneficial to have a device providing easy and accurate measurement of the refractive index with simple system configuration.

Defocusing imaging technique with a three pinhole aperture has been considered as one of advanced techniques for 3D particle tracking and 3D flow diagnostics in a microvolume [3-5]. The imaging technique uses the separation of the optical path via the three pinholes which are equilaterally aligned, and thus the separated optical path makes a spot image separated into three duplicated images when the spot isn't located on the focal plane. Since the size of the image separation depends on various parameters including the space between pinholes, fluid refractive index, and magnification, the fluid refractive index is expected to be estimated from the defocusing imaging.

Therefore, this study focuses to develop a microfluidic-based refractometry by using image defocusing method for the simple measurement of the liquid refractive index with a small sample volume (<1 μl).

2. Working principle and microfluidic device

Fig. 1 shows a schematic diagram of the working principle and the microfluidic device. When the spot pattern is on the focal plane, the single focused spot image is produced on the image plane. On the other hands, the spot pattern is not on the focal plane, the three separated spot images are produced on the image plane due to the separated optical path via the three pinhole aperture. Since the depth of the sample fluid, the space between pinholes, and the optical system are fixed, the distance among the three separated images depends on the refractive index value of the sample fluid. Thus, the refractive index can be estimated from the size of the three spot images. For the automated self-calibration, the microfluidic device is designed to have both the measurement region and the self-calibration region, which are captured on one image frame.

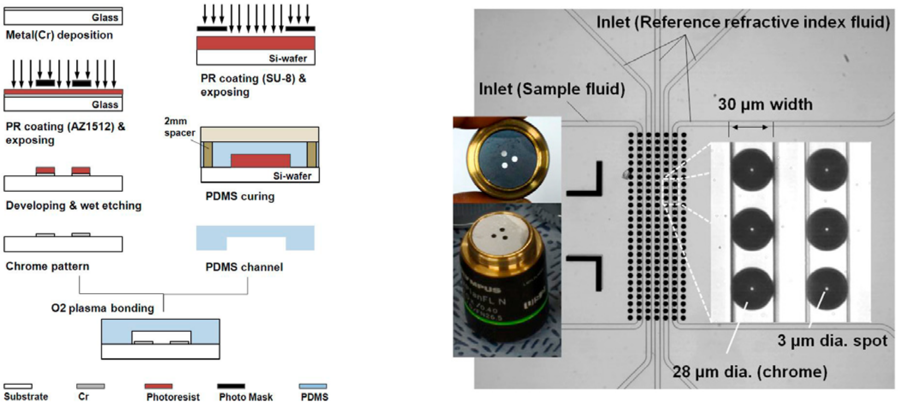


(a) (b)

Fig. 1. Schematics of (a) the working principle and (b) the microfluidic device for the refractive index measurement by using defocusing imaging technique with three pinhole aperture plate.

3. Fabrication

The fabrication procedure is illustrated in Fig. 2 (a). The microfluidic channels (Width: 30 μm, Depth: 40 μm) are prepared by a conventional PDMS (polydimethylsiloxane) replica molding technique. For the uniform thickness of the channel top, the microfluidic channel mold was covered with a glass plate attached on 2mm thickness spacers during the molding process. The bottom layer with a series of spot patterns is prepared on the glass substrate by chrome sputtering and wet etching processes. The clear spot with 3 μm diameter is patterned on the middle of the opaque chrome circle with 28 μm diameter. Fig. 2 (b) shows the fabricated microfluidic device and the aperture plate on the 20X objective lens. The microfluidic device has 5 microchannels; one is for sample fluid and the others are for reference fluids. The aperture plate with three holes was fabricated by Si-DRIE (Deep reactive-ion etching) process. The three pinholes have 1.5 mm-diameter pinholes, and equilaterally aligned. The diameter (*d*) of the circle circumscribed around the three pinholes is 4.0mm.

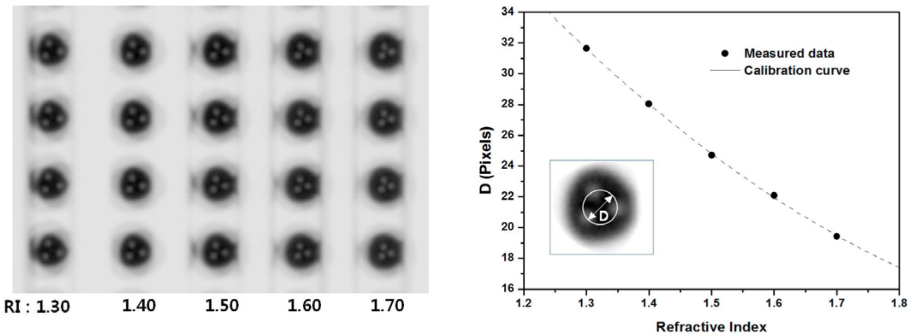


(a) (b)

Fig. 2. (a) Fabrication procedure and (b) the fabricated microfluidic device & the aperture plate with 20X objective lens (Olympus)

4. Results

The reference liquids (Cargille Labs Inc., USA) with refractive indices of 1.300, 1.400, 1.500, 1.600, and 1.700 were purchased and tested. The defocused images were captured by an inverted microscope (Olympus, Japan) and a 12bit cooled CCD Camera (Olympus, Japan). For accurate analysis, the peaks of the spot images with the sub-pixel resolution were searched with the Gaussian interpolation.



(a) (b)

Fig. 3. (a) Defocused image and (b) changes of *D* with respect to refractive indices of liquid

A defocused image of spot patterns with respect to the refractive indices is represented in Fig. 3 (a). The image defocusing is successfully obtained, and the size of the triplicated pattern decreases as the refractive index increases. The size of the image separation pattern is defined as the circumscribed circle diameter (*D*) in this study. The change of *D* with respect to the refractive indices is graphically illustrated in Fig. 3 (b). The size of the image separation, *D* is inversely proportional to the refractive index. Since the relationship between *D* and RI is not linear, a polynomial curve fitting could be used for the calibration. The quantitative comparisons between standard and measured refractive indices are represented in Table 1. The deviation between the standard and measured RI values is ranged from 0.0006 (0.04%) to 0.0036 (0.24%).

Table 1. Quantitative comparison between standard and measured refractive indices

Standard RI	Measured RI	deviation	
		RI	%
1.300	1.3006	0.0006	0.04
1.400	1.3981	0.0019	0.13
1.500	1.5036	0.0036	0.24
1.600	1.5974	0.0026	0.16
1.700	1.7008	0.0008	0.05

5. Conclusion

The proposed self-calibrated micro-refractometer with the defocusing imaging technique has been successfully developed and demonstrated with standard refractive index liquids. The maximum deviation between the standard and measured values is only 0.0036 (0.24%). Therefore, the proposed device is promising for a simple RI measurement of small fluid sample, and might be integrated with other optically functional microfluidic devices for Lab-on-a-chip applications.

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